

Effect of Growth Temperature on the Long-Chain Diols and Fatty Acids of *Thermomicrobium roseum*

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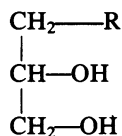
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Long-chain 1,2-diols constitute the hydrophobic backbone of membrane lipids (replacing glycerolipids) in the thermophilic eubacterium *Thermomicrobium roseum*. The effects of incubation temperature on chain length and chain branching of diols and fatty acids were investigated. The percentage of branched chains decreased, and chain length increased slightly, with increased growth temperatures.

A variety of bacteria have been shown to respond to alterations in environmental temperature by changes in the fatty acid composition of their membrane lipids. These changes have been presumed necessary in the regulation of membrane fluidity, which in turn affects membrane function. The ability to synthesize lipids, providing appropriate membrane fluidity at the temperature of growth, has been termed "homeoviscous adaptation" (20). Many organisms exhibit some form of homeoviscous adaptation (reviewed by Cosins and Sinensky [5]), although its true significance has been questioned; i.e., are homeoviscous responses truly adaptive or only a fortuitous effect of changing temperatures?

The maintenance of appropriate membrane fluidity is of particular interest in thermophilic bacteria, as membrane stability has been suggested as a major factor in the ability of microorganisms to live in very hot environments (4, 13).

Thermomicrobium roseum is an extremely thermophilic, gram-negative eubacterium (optimal growth temperature of about 70°C) (8). It has an atypical cell wall lacking any significant amount of peptidoglycan (15). The membrane lipids of this unusual organism were investigated, and a series of straight-chain and methyl-branched long-chain 1,2-diols was found (18). The diol-based lipids of *T. roseum* replace the usual glycerol-based lipids and can be considered the structural equivalent of a glycerol molecule plus one fatty acyl, aldehyde, or alcohol side chain:



Straight-chain and methyl-branched fatty acids were also shown to be present in the membrane lipids of *T. roseum* (18). With the fatty acid ester-linked to the diol (or possibly, amide linked via a polar head group), the acylated diol could provide the double-chain form typical of glycerol-based lipids.

In the present study we undertook to investigate the effect of growth temperature on the diols and fatty acids of *T. roseum*. *T. roseum* ATCC 27502 was grown in liquid medium

655 (American Type Culture Collection) at pH 8.5. Growth curves were determined in triplicate for each temperature (60, 65, 70, and 75°C), and cells for lipid analysis were harvested from one 2-liter batch in the late log phase. Total lipids were extracted from the wet cell pellet (after centrifugation to recover cells) by a modification of the method of Bligh and Dyer (3).

The diols and fatty acids were recovered from acid methanolysis of the total lipids as described previously (10, 18). The fatty acid ester distribution was determined by gas chromatography (18) against standards. Diols were analyzed as their alkane derivatives (10) and identified by gas chromatography (18) against alkane standards and against known mixtures of diols converted to alkanes. Diols were also analyzed as their acetate derivatives (9).

The effect of increased temperature on the distribution of diols and of fatty acids is shown in Tables 1 and 2. The results indicate that *T. roseum* responds to increased temperatures primarily by decreasing the ratio of branched to straight chains, although there is also a small increase in average chain length with temperature (Table 3).

The internal methyl branching on both diols and fatty acids is somewhat unusual, although 10-methylstearic acid

TABLE 1. Percent distribution of diols versus incubation temperature in *T. roseum*^a

Chain	% Distribution of diols at the following incubation temp:			
	60°C	65°C	70°C	75°C
X-methyl-C ₁₈ ^b	0.6	tr	tr	0
nC ₁₉	11.1	11.8	11.8	5.8
13-Methyl-C ₁₉	22.2	14.8	15.2	5.6
nC ₂₀	9.2	6.5	6.9	8.1
13-Methyl-C ₂₀ ^c	7.9	2.7	2.7	2.7
nC ₂₁	41.1	56.6	55.4	69.4
15-Methyl-C ₂₁	6.4	5.3	5.6	4.2
nC ₂₂	0.8	0.7	0.7	2.5
nC ₂₃	0	0.8	0.7	1.5

^a Determined as the alkane derivatives by gas chromatography. Trace amounts (less than 0.5%) of nC₁₆, nC₁₇, 11-methyl-C₁₇, and nC₁₈ chains were also detected. Diols were also analyzed as acetate derivatives; the percent composition was essentially the same as that for the alkane derivatives.

^b The exact placement of the methyl branch for this component has not been established.

^c Also includes a small amount of a 15-methyl-C₂₀ chain (as determined by mass spectrometry) that was not resolved by gas chromatography.

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TABLE 2. Percent distribution of fatty acids versus incubation temperature in *T. roseum*^a

Chain	% Distribution of fatty acids at the following incubation temp:			
	60°C	65°C	70°C	75°C
<i>n</i> C ₁₆	0.5	1.5	1.2	1.1
10-Methyl-C ₁₆	0.9	2.0	1.5	tr
<i>n</i> C ₁₇	tr	tr	0.5	tr
10-Methyl-C ₁₇ ^b	1.7	1.2	0.7	1.1
<i>n</i> C ₁₈	15.1	23.6	24.2	22.0
12-Methyl-C ₁₈	66.9	57.9	57.3	46.0
<i>n</i> C ₁₉	3.2	2.2	2.6	4.8
12-Methyl-C ₁₉	4.9	2.1	1.9	3.8
<i>n</i> C ₂₀	5.3	7.6	8.4	18.4
14-Methyl-C ₂₀	1.2	1.0	1.2	1.5

^a Determined as fatty acid methyl esters by gas chromatography. Trace amounts (less than 0.5%) of *n*C₁₄ and *n*C₁₅ chains were also detected.

^b Also includes a 12-methyl-C₁₇ chain (as determined by mass spectrometry) that was not resolved by gas chromatography.

(tuberculostearic acid) is found in mycobacteria (12). Methyl-branched-chain fatty acids are common in thermophilic eubacteria (11), but the branches are generally in the *iso* or *anteiso* position, especially the former (16).

Thermophiles may respond to increased growth temperature by increasing average fatty acid chain length (7, 17, 21), by increasing the proportion of straight to branched-chain fatty acids, or by increasing the ratio of *iso*- to *anteiso*-branched fatty acids (2, 14, 16, 19, 22). The longer the chain the higher the melting point, and straight chains have higher melting points relative to their branched-chain counterparts. This difference is very small, however, for *iso*-branched chains; on the other hand, the melting points of *anteiso*-branched chains are substantially lowered, as is the melting point of tuberculostearic acid (13.2°C) compared with stearic acid (69.6°C) (6).

The internal methyl branches of both diols and fatty acids are presumed to substantially lower the chain melting point, in analogy to *anteiso*-branched fatty acids and tuberculostearic acid. Thus at higher growth temperatures, the proportion of the lower-melting-point chains is reduced.

It is not clear why an extreme thermophile would have a relatively high percentage of internal branched-chain hydrocarbons in its membrane lipids. It has been suggested that the membrane lipid transition from the gel phase to the liquid-crystalline phase determines the lower, but not the upper, temperature limits for growth (1, 14). It may be more important, therefore, for a microorganism to be able to

respond to decreased rather than to increased growth temperatures, at least within the limits where membrane stability is a deciding factor.

Confirmation of the effect of internally branched hydrocarbon chains on membrane fluidity must await biophysical studies. An equally important point, however, concerns diol function. These hydrocarbons are so far unique (in membrane lipids) to *T. roseum*. The evidence here indicates that both diols and fatty acids undergo similar changes in branched-chain composition and in chain length with alteration in temperature. This strengthens the hypothesis that the acylated diol (with the polar head group also attached) is functionally equivalent to glycerol-based membrane lipids, the diol tail acting as the second fatty acid of a diglyceride.

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TABLE 3. Effect of temperature on the straight chain/branched chain ratio and on the average chain length of diols and fatty acids in *T. roseum*

Incubation temp (°C)	Compounds	% Straight chains	% Branched chains	Avg chain length ^a
60	Diols	62.2	37.1	20.15
65		76.4	22.8	20.39
70		75.5	23.8	20.38
75		87.3	12.5	20.72
60	Fatty acids	24.1	75.6	18.17
65		34.9	64.2	18.13
70		36.9	62.6	18.17
75		46.3	52.4	18.46

^a Chain length refers to the number of carbons, excluding methyl branches.

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